COMPLEMENT-FIXATION IN TUBERCULOSIS *

H. J. CORPER

From the Laboratories of the Municipal Tuberculosis Sanitarium of the City of Chicago

The discovery of the tubercle bacillus by Koch in 1882 placed the diagnosis of tuberculosis on a substantial foundation, but the bacillus cannot always be demonstrated early in the discharges, frequently never appearing, and even if present giving little clue to the activity or inactivity of the disease. Clinical findings give us information regarding activity or inactivity only in a crude way. Thus far biologic methods of diagnosis have been of little practical value with one exception—complement-fixation. Tho not fulfilling the early expectations, this method of diagnosis is gradually being improved so that there is promise of its becoming as valuable a diagnostic test as the Wassermann in syphilis.

HISTORICAL REVIEW

The first application of complement-fixation in tuberculosis was made by Widal and LeSourd[1] in 1901. They obtained deviation of complement in certain cases of tuberculosis, using as antigen homogeneous emulsions of tubercle bacilli of the Arloing-Courmont strain. In 1903 Bordet and Gengou[2] demonstrated the presence of antibody capable of uniting with tubercle bacilli and fixing complement in the sera of tuberculous animals. Wassermann and Brück[3] in 1906 demonstrated the presence of an antibody to tuberculin in patients treated with tuberculin, but they examined only 13 cases of pulmonary tuberculosis. Caulfield[4] in 1911 examined 104 cases of pulmonary tuberculosis with bacillary emulsion as antigen and obtained 33% Turban I cases, 70% Turban II, and 62% Turban III positive results. Laird[5] (1912) out of 84 tests in 34 cases obtained 24 positives in 4 cases, using watery emulsion of tubercle bacilli (which he does not describe); his results were inconclusive. Hammer,[6] using O. T. and extracted tuberculous nodules, obtained 97% positive results in 46 tuberculous cases. Calmette and Massol,[7] using preparations made from tubercle bacilli by extracting with water and peptone, obtained in 134 cases 92.5% fixation. Fraser[8] (1913), testing a large variety of antigens, found that living bacilli gave no fixation in 96.6% of normal individuals, but gave positive reactions in 42.3% of tuberculous individuals. She states that the most reliable antigen

* Received for publication March 2, 1916.

1 Cited by Shennan and Miller, Edinburgh Med. Jour., 1913, 10, p. 81.
2 Compt. rend. Acad. de sc., 1903, 137, p. 351.
3 Deutsch. med. Wchnschr., 1906, 32, p. 449.
4 Jour. Med. Research, 1911, 24, p. 122.
5 Ibid., 1912, 27, p. 163.
6 München. med. Wchnschr., 1912, 59, p. 1750.
7 Compt. rend. Soc. de biol., 1912, 73, p. 120.
8 Ztschr. f. Immunitätsf., 1913, 20, p. 291.

is prepared from living human bacilli, and that diagnostically the complement-fixation test with living bacilli is of more value from the standpoint of positive results than any other reaction discovered to date. She believes the absence of antibodies accounts for the low percentage of results obtained. Dudgeon, Meek, and Weir[9] also tested a large number of antigens, and in 102 untreated cases obtained 86 positive results, while all cases which had been treated with tuberculin gave positive results. Products of the bacilli themselves were found to be the most satisfactory as antigen. With an alcoholic antigen[10] prepared from tubercle bacilli they obtained from a total of 234 cases, 209 (89.3%) positives, 194 of these on 1st examination, 11 (of the 15 negative) on 2nd examination, and 4 more on 3rd examination. Besredka[11] (1913) prepared an antigen by growing tubercle bacilli (of a questionable nature since they grow in from 24 to 48 hours) on egg broth, heating it, and filtering. With this antigen Bronfenbrenner[12] (1914) obtained a very high percentage of positive results, 93.8% in active cases, and 55.5% in convalescents, while suspected cases gave 75% and syphilitic sera 24% positive reactions. Inman,[13] and Kuss, Leredde, and Rubenstein[14] found this antigen nonspecific. McIntosh, Fildes, and Radcliffe[15] (1914) also justly criticized Besredka's antigen and concluded, after testing a large number of antigens, that the living bacillary emulsion was best, yielding 76.7% positive results in 43 definite cases of phthisis, 80.7% in surgical tuberculosis, and 37.5% in glandular tuberculosis. Of 87 normal individuals only 3 gave positive reactions (2 of these were lepers and 1 had Addison's disease). Negative reactions were obtained in 18 syphilitic patients. They look upon a positive reaction as indicative of active tuberculosis. Stimson[16] (1915), who gives a fairly exhaustive table of the recent literature, reports a small number of cases, in which a variety of antigens were used, but his results were inconclusive. Craig[17] (1915) reports the results of examination of 166 cases of pulmonary tuberculosis, in which he employed as antigen an alcoholic extract of several strains of human tubercle bacilli which had been grown on a liquid medium of alkaline broth containing egg; 96.2% positive results were obtained in active cases and 66.1% positive in inactive cases. One hundred fifty cases of syphilis gave only 2 positive reactions and these on further examination revealed lesions in the lungs. One hundred other diseases examined all gave negative results. It remains, however, for future investigators to corroborate Craig's findings by proving this antigen to be specific.

The most reliable investigators concede that a suspension of living tubercle bacilli is the only one of the many antigens used, that is of specific value. The objections to the bacillary emulsion are the small leeway between the antigenic and the anticomplementary dose, the turbidity produced in the tubes, and the fairly high percentage of nonspecific reactions. In the hope of overcoming these difficulties, it was

[9] Lancet, 1913, 184, p. 19.
[10] Jour. Hyg., 1914, 14, pp. 52, 72.
[11] Compt. rend. Acad. d. sc., 1913, 156, p. 1633.
[12] Arch. Int. Med., 1914, 14, p. 786.
[13] Compt. rend. Soc. de biol., 1914, 76, p. 251.
[14] Ibid., p. 244.
[15] Lancet, 1914, 185, p. 485.
[16] Bull. Hyg. Lab. U. S. P. H. and M.-H. S., 1915, No. 101, p. 7.
[17] Am. Jour. Med. Sc., 1915, 150, p 781.

decided to try to obtain the antigen from the tubercle bacillus by processes as nearly identical with those that occur in the body as possible. With this in mind, and with the realization that bacterial antigens are probably of protein character, the following investigations were carried out.

To determine the most favorable condition for the liberation of the antigenic products from the tubercle bacillus, heavy suspensions of living tubercle bacilli of human origin were made in sterile tubes with sterile physiologic salt solution. One set of tubes was incubated, another set was kept at room temperature, and a third, as control, heated for 30 minutes in a boiling water bath to kill the bacilli, and then incubated. The disintegration of the bacilli was observed in the amount of noncoagulable nitrogen liberated, determined by the Folin micro method. A typical set of these results is plotted in Chart 1. (The complete experimental data, methods, and results will be reported in a subsequent paper.) As noted in Chart 1, there was a gradual liberation of noncoagulable nitrogenous substances from the tubercle bacillus at incubator temperature, reaching its maximum at about the 8th day. This did not take place after the bacilli had been killed by heat. At room temperature it did not occur to any appreciable extent within 10 days.

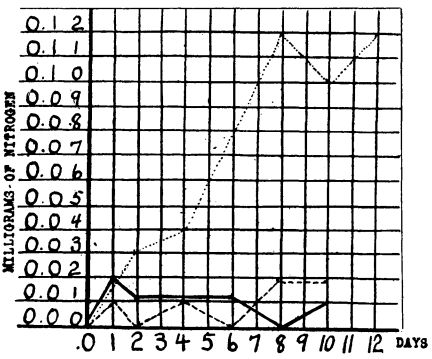

Chart 1

——————— The heated and incubated control.
..... The incubated aseptic.
– – – – – The room-temperature aseptic.

The process by which these nitrogenous materials appeared was next studied. It is of course conceivable that it might be either a simple dissolving out of endogenous nitrogenous materials from the bacilli, or the result of enzyme action, an autolysis. As shown by Wells and Corper[18] toluene destroys the tubercle bacilli but leaves the enzymes intact. Chart 2 gives the results of an aseptic (suspension of bacilli in salt solution without the addition of an antiseptic) and antiseptic (toluene) experiment carried out at incubator temperature. Autolysis of tubercle bacilli was perceptible at the 2nd or 3rd day, and reached a maximum at the 6th or 8th day at incubator temperature. These experiments were repeated with bovine tubercle bacilli, and the same found to hold true.

The next question was whether or not this autolysis bears any relation to the increase of antigenic strength in the autolysate. In order to test this, the autolysate from a series of suspensions of tubercle bacilli in sterile physiologic salt solution was tested at definite intervals for noncoagulable nitrogen content, and coincidently titrated for antigenic strength. Varying amounts of the antigen were titrated against a four-plus tuberculosis serum and the amount of complement-fixation noted. As seen from Table 1, altho the nitrogen figures and the antigenic titer do not show an exactly parallel increase, they do bear a certain relation to each other, and it is to be noted that an antigenic

[18] Jour. Infect. Dis., 1912, 11, p. 288.

titer of 0.1 c.c. on the 1st day became gradually and consistently a titer of 0.001 c.c. on the 6th day. Thus the autolysate from suspensions of living virulent tubercle bacilli grew stronger in antigenic titer coincidently with the occurrence of autolysis.

In order to test the value of the autolysate antigen it was compared in a large series of cases (over 600) with the bacillary emulsion. In a general way it can be stated that the autolysate antigen possesses the following advantages over the bacillary emulsion: it has a much larger range between the antigenic and anticomplementary doses (even 0.2 c.c. of a 0.001-c.c. strength has no anticomplementary effect); it does not lose its titer when kept on ice (several autolysates have kept their titers for 4 months); it produces no interfering turbidity in the hemolytic system; and it is more specific than the bacillary emulsion.

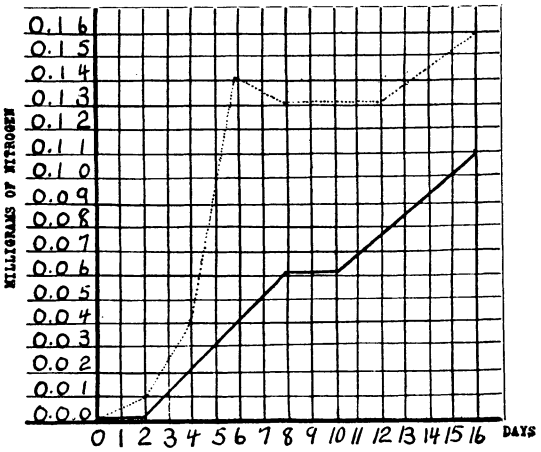

Chart 2

———— Aseptic autolysis in incubator.
..... Antiseptic (toluene) autolysis in incubator.

More than 600 examinations were made with the bacillary emulsion and the autolysate as antigens, but for the sake of accurate comparison only the results from 361 cases are given in tabulated form, only such cases being included as had been accurately worked up from the clinical standpoint. If there existed any question as to the accuracy of diagnosis, or if all the clinical facts did not agree, the case was discarded. The results obtained are given in Table 2. The cases are divided according to the classification adopted by the National Association.

If a single plus is used as criterion, a higher percentage of positive results was obtained in the nontuberculous and the more advanced

TABLE 1

CORRELATION OF AUTOLYSIS AND ANTIGEN-FORMATION

Nitrogen Curve		Antigen Curve							
Days	Mg. N. per c.c.	0.2 c.c.	0.1 c.c.	0.5 c.c.	0.01 c.c.	0.005 c.c.	0.001 c.c.	0.0005 c.c.	0.0001 c.c.
0	0.00	++++	++++	+++	—	—	—	—	—
1	0.00	++++	++++	+++	±	—	—	—	—
2	0.01	++++	++++	++++	+++	±	—	—	—
3	0.02	++++	++++	++++	++++	+++	++	+	—
4	0.03	++++	++++	++++	++++	++++	+++	+	±
6	0.05	++++	++++	++++	++++	++++	++++	++	++
8	0.06	++++	++++	++++	++++	++++	++++	+++	++
10	0.08	++++	++++	++++	++++	++++	++++	+++	+
13	0.15	++++	++++	++++	++++	++++	++++	+++	++

TABLE 2

RESULTS OF COMPLEMENT-FIXATION TESTS ACCORDING TO THE ONE-PLUS (PLAIN TYPE) AND THE DOUBLE-PLUS (BOLD FACE TYPE) CRITERIONS

Cases	Number of Patients Examined	Number of Examinations	Negative with Bacillary Emulsion	Negative with Autolysate	Positive with Bacillary Emulsion	Positive with Autolysate	Tubercle Bacilli in Sputum	Results of von Pirquet Tests
Nontuberculous...	25	26	(1 A)* 19 **23**	19 **24**	6 **2†**	7 **2†**	25—	1-11+
Questioned nontuberculous.....	11	11	5 **6**	7 **9**	6 **5**	4 **2**	11—	3-6+
Incipient inactive.	47	50	(2 A) 30 **39**	35 **43**	18 **9**	15 **7**	46-1+	
Incipient active...	27	30	(5 A) 10 **18**	14 **23**	15 **7**	16 **7**	19-7+	
Moderately advanced inactive.	12	14	8 **10**	9 **13**	6 **4**	5 **1**	6-6+	
Moderately advanced active...	47	55	(8 A) 13 **24**	27 **37**	34 **23**	28 **18**	12-34+	
Far advanced inactive.....	5	5	3 **4**	3 **4**	2 **1**	2 **1**	3-2+	
Far advanced active.....	187	216	(28 A) 81 **112**	(1 A) 110 **155**	107 **76**	105 **60**	15-172+	
Totals.....	361	407	213 **280**	225 **309**	194 **127**	182 **98**		

* A = an anticomplementary result.

† One of these cases had received a tuberculin injection for diagnostic purposes a short time previous to drawing the blood.

cases. The double-plus criterion, however, seems to give the more accurate view of the state of affairs, giving as it does a low percentage positive in normals, tho it also lowers the number of positive findings in the clinically certain tuberculosis cases. The results under the double plus may be summed up as follows:

1. Only 1 nontuberculous case out of 25 gave a positive reaction (96% negative by both tests). The second positive had received a tuberculin injection a short time before the test.

2. Of questionable nontuberculous cases 18% were positive in the autolysate test, 45.5% in the emulsion test.

3. Incipient inactive cases gave 14% positive with the autolysate and 18% with the emulsion.

4. Incipient active cases gave the same result with both, 23.3% positive.

5. Moderately advanced inactive cases gave 7.15% positive with the autolysate and 28.6% with the emulsion, while the active cases gave 37.7% with the autolysate and 41.8% with the emulsion.

6. The far-advanced inactive cases gave 20% positive in both tests, while the active cases gave 27.6% with the autolysate and 35% with the emulsion.

A greater percentage of reactions was always obtained in the active cases, but the results seem to indicate, as pointed out by Fraser,[8] that antibodies in free form capable of binding antigen are apparently not always present in the sera of tuberculous individuals, but are most liable to be present in the active form of the disease. (It is significant that all von Pirquet negatives were negative also as regards complement-fixation.) Has complement-fixation, then, any practical value as a diagnostic test for tuberculosis? It can be answered that in conjunction with other findings, complement-fixation makes the diagnosis of tuberculosis definite. It is of value also from a differential diagnostic standpoint in that it points out tuberculosis, when positive, as against syphilis, abscess of the lung, empyema from other causes, carcinoma, bronchiectasis, etc.

Now that the complement-fixation test has been found lacking in point of percentage efficiency as a diagnostic test for tuberculosis, the question arises as to whether or not it is possible by further study to make the test more efficient. With a view to obtaining a higher percentage of positive results a number of autolysates prepared from different strains of virulent tubercle bacilli are being tested on the same sera in order to prove whether or not a polyvalent autolysate antigen

would be more efficient than a monovalent one; the sera are being drawn at various intervals during the day to see whether there is an especially opportune time for obtaining the antibodies in the sera, as suggested by the periodicity of the temperature curve. Weekly intervals are also being considered. Finally, antigen and antibody tests are being made coincidently on the same sera, as it has been suggested that possibly in the absence of antibodies a test for antigen may give results.

SUMMARY

Virulent cultures of tubercle bacilli free from all foreign substances suspended in sterile salt solution undergo autolysis at incubator temperature as indicated by the liberation of nitrogenous substances, the autolysis reaching a maximum from the 6th to the 8th day.

During the autolysis of virulent cultures of tubercle bacilli there is a coincident liberation of antigenic substances which possess advantages over a suspension of living virulent tubercle bacilli as antigen for complement-fixation tests in tuberculosis.

The examination of 361 persons (25 of them normal, 11 questionably nontuberculous, and 325 definitely tuberculous), using both an emulsion and an autolysate prepared from living virulent human tubercle bacilli as antigens, shows that—

(a) The complement-fixation test for tuberculosis is not absolute, being positive only in about 30% of all the clinically definite cases of tuberculosis both active and inactive. Active cases give a higher percentage of positive results than inactive cases.

(b) The value of the complement-fixation test for tuberculosis lies in the fact that, taken in conjunction with other findings, a definitely positive reaction makes the diagnosis of tuberculosis certain.

(c) It is of value also from a differential diagnostic standpoint in that it indicates tuberculosis, when positive, as against syphilis, carcinoma, abscess of the lung, empyema from other causes, bronchiectasis, etc.

The practical absence of a reaction in nontuberculous cases makes this test, when positive, of far greater value in the diagnosis of tuberculosis than any of the biologic tests for tuberculosis thus far discovered. A positive test was never obtained in the absence of a positive von Pirquet reaction, but a large percentage of clinically normal individuals giving positive von Pirquet reactions were negative in fixation tests.